Morphological and Chemical Studies of Developing Cerebral Cortex in the Cat.

by

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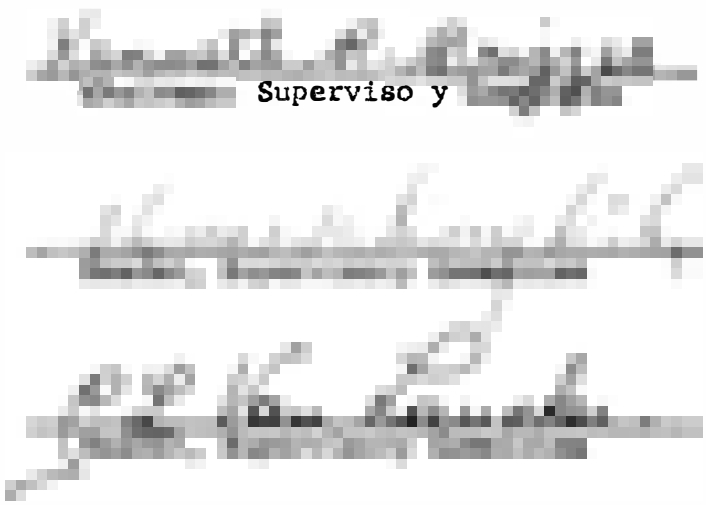

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I. INTRODUCTION

A thorough knowledge of the pattern of the developmental process in different species of animals is important as a basis for experimental work in the field of metabolism, electrophysiology and other related fields.

Most morphological studies of the process of development in the domestic cat have been based on qualitative observations rather than on quantitative determinations. Therefore there is an almost complete lack of information concerning the quantitative morphological changes in the developing cerebral cortex in this species. Since the cat is an animal universally used in biological experimental work, an accumulation of such quantitative data is of great importance.

The purpose of this thesis is to study some of the quantitative aspects of changes occuring in the cerebral cortex of the cat during the process of development and to establish the chronological relationship between the chemical and structural changes.

It is hoped that the data presented here may serve as a frame of reference to investigators engaged in experimental research carried out on the cat.

II. HISTORICAL REVIEW OF LITERATURE

A. The Quantitative morphological analysis of the cerebral cortex.

The second half of the past century was a beginning of the great "cytoarchitectonic" era in the field of neuroanatomy. The gross structures of the central nervous system and their cellular elements were studied extensively. Meinert (1867) was the first who calculated the number of the cells in the human cerebral cortex. The value obtained by him was low as compared to more recent data. In 1898 Nissl observed that the cerebral cortex of lower animals contains more cells for a given volume of tissue than that of the animal with a higher organization of the cerebral cortex. Inspite of the rapid progress in the studies in the field of qualitative morphology, no criteria had been worked out for the quantitative ecaluation of the differences between the species, until V. Economo and Koskinas ('25) introduced the concept of the gray/cell coefficient. According to the definition of von Economo the gray/cell coefficient is the ratio between the mass of "griseum" and the total mass of the cells contained in the "griseum" (under the "griseum" von Economo understands the entire entity of the cellular elements, interstitial substance and the blood vessels of the cerebral cortex).

The determination of the gray/cell coefficient required a determination of total cellular volume and of the density of the cells. Von Economo and Koskinas solved this problem in the following way: the sections of the cortex were photographed (magn 1 x 100) and all the cells

and parts of the cells within a square of known dimensions were measured on the photomicrograph. Thus, his concept of the gray-cell coefficient is two dimensional rather than 3 dimensional. The authors themselves admitted that their counts may be a little high. V. Economo was the first to calculate the gray/cell coefficient of the human cerebral cortex. He found it to be 27:1 (a value very low as compared with more recent data). Bok ('36) calculated the relationship between the size of nucleus and cell body volume. His formula for the cellular volume as cited by Ryzen and Campbell ('55) is $C = N^{\frac{2}{3}}$ where C is the volume of the cell body and N is nuclear volume. The volume of the nucleus was calculated by Bok as that of the sphere (calculated from measurements of the greater and lesser diameters of an Ellipsoid nucleus). Bok found that the volume of the cell increases as a quadratic function of the nucleus. He also reported that in the layers 2 and 3 of the cortex the nuclear volumes increase proportionately to the depth, and in the layers IV, V, and VI proportionately to the square of the depth.

Von Bonin ('33), ('38), ('39) worked out distribution curves for the nuclei of the cells of different sizes for different areas of the cerebral cortex of the man, Orang, and Cebus. He found that the nuclear volumes in the striate area is the largest in man (135-185 μ^3) and the smallest in cebus (78-81). He concluded that the distribution curves are different for different species. Comparing the mean volume of the nuclei in the brain of man, cebus and cat, von Bonin found the values for the man 370 μ^3, for the cebus 305 μ^3, and for the cat 441 μ^3. Von Bonin states that the difference between ordinary and giant cells of the cortex increases with increased complexity of the brain. The solitary large pyramidal cells are larger in

man than in cebus. The cells of the cat cortex are generally relatively large and the difference in the size of its constituent cells is not as significant as in the man. Von Bonin used the same method of calculation of the nuclear volume as Bok.

Agduhr ('41) subjected v. Economo's method for determination of the gray/cell coefficient to severe criticism. He pointed out that the main source of errors in v. Economo's method was the use of the photomicrographs and the low magnification at which they were taken. Agduhr points out that the differentiation between the neurons and neuroglia under these conditions is impossible, and the cells sometimes lie so near to one another on the photographs that they appear to be one cell rather than several. Agduhr also states that in the calculations of v. Economo no consideration has been paid to the fact that the same cell can appear in more sections than one. He proposed his own method involving cell counts and measurements under microscope using oil immersion. The sections were cut at 5 μ. The calculations of the cellular volumes are based on the assumption that all the cells are spheres or ellipsoids. The volumes of the cells were calculated the same way as von Bonin and Bok calculated the nuclear volumes. The height and width of the cells were used as the greater and lesser diameters, the measurements were carried out by means of a square ocular micrometer projecting small squares into the field.

In order to eliminate the errors due to the probability of counting the same cell in several sections, Agduhr introduced the use of coefficients which were different for cells of different sizes. Criticizing this method, Haug ('53) pointed out that Agduhr*s calculations were based on the assumption

that every small part of the cell can be counted in the sections, but that since this is technically not possible, the calculations are a source of error by themselves. Haug emphasizes that the assumption that all cells are spheres is another source of error.

In 1943 Chalkley published his method. In this method several hairs are attached (glued) to the ocular and various portions of the tissue, chosen at random, are brought into focus to determine the frequency with which any given structural entity is "hit". This permits the determination of the proportion of the tissue occupied by any given structural element. This method does not involve any measurements of cells and is independent of the shape of the cells, thus eliminating a major source of errors. Haug ('56) compared the data obtained by his own method and that of Chalkley. He found that the coincidence was very good and stated that the values of any two computations are nearly equal which justifies the conclusion that both Chalkley and Haug's methods are unobjectional and correct.

However, Haug found that the Chalkley method presents two difficulties: First, it presupposes a minimum of focal depth, but the limits of accommodation of the eyes of the observer change with age. Therefore, people of different age have to use different objectives - i.e. these most suited for one's limits of accommodation. Second, since the neurons do not have any discernible cell membrane, it is difficult to decide whether or not a certain part of the cell is actually "hit" by the hair.

Another method of Chalkley ('49) is concerned with the determination of cell density. According to this method the cells are counted within the cylinder "cut out" by focusing down into the section a circle placed in the

eye-piece of the microscope.

Shariff ('53) using Chalkley's method determined the gray/cell coefficient in man, chimpanzee, cercopitheous, hapale and tarsius. He found that the mean cell volume decreases from man to tarsius. (The cellular volume for man in the agranular cortex is 2,468, chimpanzee - 1035 and tarsius - 580.) The density increases and the inverted gray/cell coefficient (calculated as the total cell volume/volume of gray) decreases. For the determination of density Shariff used the planimetric method measuring the volume of the cortex (the sections projected on paper at a magnification of 3x were drawn with hard pencil and their surface measured with a planimeter. By the multiplication of the surface by the thickness of the section the volume of the section was determined.)

Haug ('53) published his method for determining the gray/cell coefficient, which is extensively used by him and his followers at the present time. Haug*s method involves the differential cell counts and measurements of the cells in thick, thionin stained sections. The counts and measurements are carried out under microscope using oil immersion and an ocular grid of known dimensions. Only the width and height of the cell are measured. The volume of the pyramidal cell is calculated from the formula for the cone, ($F = \frac{\pi (\frac{B}{2})^2 H}{3}$ where B is width, and H - the height of the cell) the volume, of Spindle-shaped cells from the formular for the cone multiplied by two ($F = \frac{2\pi (\frac{B}{2})^2 \frac{H}{2}}{3}$), the volume for the spherical cells from the formula for the sphere ($F = \frac{4}{3}\pi (\frac{B}{2})^2$). Haug counts all the neurons, whole cells and parts of the cells as long as they do not lose 25 - 50% of their height. Since portions of the same cells may be included in several sections the

total volume of the neurons counted may be too great, to correct for this Haug calculated curves of coefficients (one for the smallest cells and the other for all others), by which the total volume of the cells is multipled. The coefficient depends on the ratio of cell width/thickness of the section. The determination of cell density Haug based on the "cortical cylinder." As he stated ('53): "In order to eliminate the evasive concept of the cortical depth, I am using the concept of the cortical cylinder. A cylinder stands on the upper surface of the cortex. In this cylinder I determined the gray/cell coefficients of the single layers". (It is the opinion of the author of this thesis that Haug's "concept of the cylinder" is not better than the concept of the "cortical depth").

Haug used his method in phylogenetic studies comparing the cerebral cortices of the man, horse, sheep and rabbit. He found that the gray/cell coefficients are different for different cortical regions of the same animal and that in the animals with higher degrees of organization of the cerebral cortex, the cells tend to differentiate into smaller and larger ones, while the cell density tends to diminish. In some cases the process of phylogenesis can be different. The cortex of the horse has a relatively high cell density while the cells are not well differentiated. The gray/cell coefficient has been found higher in the layers II IV and VI while in layers V and III it is relatively lower.

Tower ('54) studied the cellular density of the cerebral cortex of the whale and compared it with that of the cortex of the Indian elephant. He found that density is (6,500 - 7,100 cells/mm^3) in the same range in the fin whale as in the elephant (6,900 cells/mm^3). Tower used the following

method of cell counts. All the cells contained in 1 mm^2 of a 20 μ section were counted and their number multiplied by 50 to obtain the density per mm^3. He also compared the cell density of the cortices of different animals starting with the mouse and concluding with the elephant and whale. He found that the cell density of the cortex decreases proportionately to the increase of weight of the brain.

Ryzen and Campbell used Chalkley's method in their studies of the cerebral cortex of the Sorex pacificus ('55). The data obtained by this method agreed well with those obtained by measurements of nuclei (the cells were focused on ground glass and drawn on tracing paper). Using Bok's formula for the determination of the cellular volume, these workers found that in all neocortical regions the average nuclear volume decreases within the upper and lower cell groups (the nuclear volumes continually form 3 distinct groups: upper, lower and the deep group). The sensory region contains relatively more cells in upper layers while the motor cortex has more cells in the lower. They found that small cells prevale in the sensory region while large cells are prominent in the motor region. The cell density increases as the volume increases.

B. Developmental Studies.

The method of quantitative morphological analysis has been used relatively little in the developmental studies.

Peters and Flexner (1950) studied the prenatal stage of the development of the cerebral cortex in the guinea pig using Chalkley's methods (1943) and (1949). The number of observations taken within each

layer of the cortex was proportional to the depth of the cortex. These workers found that there is a critical period during the fetal stage of development of guinea pigs between the 41st and 45th day of gestation. During this period the nuclear volume reaches the value of the nucleus in the adult guinea pig, the cell processes increase rapidly and the activity of adenyl pirophosphatase increases. All the layers of the cortex are present at this time. Peters and Flexner think that during this period many neuroblasts differentiate into neurons. The cell density decreases steadily from the 30th day of gestation to the term. The cellular volume increases reaching a size close to that of the nucleus of the adult guinea pig.

Brody (1956) using Bok's method studied quantitative changes in the human cerebral cortex throughout life from new born to 95 years of age the cell counts were carried out on 10 μ thick sections and only on the side of the block within the sulcus (wall). Sections on which there was no block large enough along the sulvus were discarded. Using a grid reticule all the cells were counted in the block at a magnification of 430. His data indicate that the mean cellular volume was higher in the adult than in the infant, but no significant changes have been observed in the cellular volumes of the older groups. The cortex of the infants in all specimens had the highest cell contents per unit of the cortical volume. The number of cells did not seem to increase after birth, therefore Brody thinks that the decrease of density is due to the increased glial and nerve cell processes, increase in myelin and exogenous elements, like nerve fibers, blood vessels, spaces and others. He also observed that the smaller the

size of the cells the higher is their density.

As far as the author of this thesis knows, no quantitative studies of the morphological changes during the period of development of the cat has been done.

Tilney ('31) studied qualitative the morphological changes in the cortex during the prenatal development of the cat, from the time when the cortex starts forming to the 120 mm fetus. He found that the "cortical zone" (primordium of the granular layer), is formed at the 13 mm stage from the cells which migrated from the ependymal zone. The cell nuclei at this time are more basophilic than those of the mantle zone and their cytoplasm is a little more extensive.

In the 50 mm stage the cells of the cortical zone are larger. Some of them have large vesicular nuclei, but the cytoplasm is scanty. The cells are arranged in cords perpendicular to the surface of the cortex Secondary lamination starts at this stage: several rows of cells, similar to those of the granular layer are found just below the granular layer.

Tilney defined the layers of cortex in the 12 mm stage as: ependymal, mantle, internal granular, pyramidal, external granular and marginal.

C. The Nucleic Acids

a) The Nucleic acids and their part in the process of development.

The nucleic acids were discovered in the animal cells by Miescher (1841) and in the plant cells by Altmann (1889). Jones ('20) stated: "There are but two nucleic acids in nature, one obtainable from the nuclei

of the animal cells, and the other from the nuclei of the plant cells". Until the early thirties, it has been thought that the PNA is the nucleic acid of the plants while the DNA is found exclusively in the animal nuclei.

Brachet ('31) expressed the belief that the eggs have a cytoplasmic reserve of PNA. He ('33) has shown that the feulgen reaction was negative in the unfertilized eggs, and the eggs just fertilized, while after the beginning of development it became positive showing large amounts of the DNA in the nuclei of the developing cells.

On the other hand it has been found that when the cells are cynthesising protein the PNA is present in large amounts (Brachet-1941, Casperson-1941).

Caspersson (1933), Caspersson and Schultz (1938, 1939) found accumulations of the DNA in the chromosomes during the prophase. Caspersson and Schultz (1939) found high concentrations of PNA in the zone of growth of the tip of the root of allium, but they did not find much PNA at the base of the root.

Since the formation of protein is quantitatively the main process in the growing cell, and the amount of PNA in the cytoplasm during this process is high Caspersson and Schultz ('40) concluded that the PNA is involved in protein synthesis. Hyden ('43) found gradient concentration of basic proteins from the nucleolus towards the part of the nuclear membrane where the formation of PNA takes place, indicating a migration of basic proteins from the nucleolus to the nuclear membrane.

Thorell ('44) found that the basic protein substances in the nucleolus were rich in diamino acids.

Discussing the part played by nucleic acids in the process of growth ('47), he points out that during the embryonic growth in the earliest stages of hemopoiesis the blood cells have a Feulgen-negative nucleolus, surrounded by an outer layer of nucleolus associated chromatin.

In the later, more mature stages only a small chromocenter in the nucleolus can be found.

From the chemical changes in the RBC during the period of development it seems that only during and prior to the principal increase in the cell substance, the cell contains a large PNA containing nucleolar mass and high PNA concentrations in the cytoplasm. Thorell states: "During the first phase-the growth phase-it is principally the formation of the basic cell protein substances which takes place. The next phase involves a decline in the growth process, cytochemically observed as a rapid decrease in the concentration of the cytoplasmic ribose nucleic acids and the nucleolar mass. After this, the first processes which lead up to differention of the cellular proteins appear".

Davidson ('47) found the ratio PNA/DNA in adult liver of the rabbit was twice as high as that of the liver of the embryo. Flexner and Flexner ('50) found that the ratio PNA/DNA increased about six times during the 28th day of gestation of the guinea pig to the adult animal. They also observed a steady decrease of the mean levels of PNA and DNA during the fetal period. At term the levels of PNA and DNA were slightly higher than in the adult guinea pigs.

There are suggestions in the data of some of the more recent researchers that the DNA is more active in the protein synthesis rather than the PNA: Gale ('55) on the basis of experiments with staphylococcal cells

states: "Removal of nucleic acids from disrupted staphylococcal results in a marked decrease in the incorporation of certain amino acids and the incorporation can be restored by addition of staphylococcal nucleic acid to the incubation mixture. Inspections of the time course of incorporation in the presence or absence of added nucleic acid show that both DNA and PNA restore incorporation but that DNA is more effective than PNA and that the amount of incorporation attained at equilibrium is higher with DNA than with PNA". Gale's data are still under dispute.

Koenig ('58) observed that adenine-8-C-14 and orotic-6-C-14 acid injected intrathecally were rapidly incorporated into pentose nucleic acid of the central nervous system. PNA exhibited a high specific activity after the first injection and the DNA very little. After repeated injections DNA exhibited high specific activity, but as Koenig himself states this by itself does not prove definitely protein synthesis by DNA.

b) Methods for the Determination of the Nucleic Acids.

There are various methods for determination of the nucleic acids but only the most popular of them will be reviewed here.

The most popular methods at the present ime are:

Caspersson ('36) worked out a microspectrophotographic method for the determination of the nucleic acids in the central region of ultraviolet light. It is based on the high selective absorption of light by the nucleic acids in the central u-v region (2600Å) - due to the puring and pyrimidine bases in their molecule.

The x-ray method worked out by Engstrom ('46) is more sensitive in the phosphorous determination than are the colorimetric procedure.

The method of Schmidt and Thannhauser ('45) is a chemical method, based on the separation of the PNA and DNA by means of incubation for 15 hours with IN NaOH at 37°C and determination of phosphorus in both fractions.

This method has been extensively used during the lase decade.

Schneider's method ('45) is based on extraction of the nucleic acids with heated TCA and determination of the PNA and DNA without separation. The content of PNA and DNA is determined by the orcinol color reaction in an aliquot of extract, and the content of DNA is determined by the color reaction with diphenylamine or with cysteine in another aliquot. The amount of PNA is calculated as the difference between PNA + DNA and the content of the DNA.

Feulgen and Rossenbeck ('24) introduced a histochemical method for the determination of DNA in histological preparations by staining the sections with Shiff reagent - solution of basic fuchsin reduced with sodium bysulfite. The chemical nature of Feulgen, or nuclear reaction, is not well understood.

Concluding the review of the literature the author would like to point out, that because of the important part played by the nucleic acids in the synthesis of proteins during the period of development, in the determination of PNA and DNA during this period is very important.

III MATERIALS AND METHODS

A. Materials.

The data presented here were obtained as a result of morphological and chemical studies of the suprasylvian gyrus of a total number of 101 cat brains. The animals used for the histological studies were of 1, 3, 5, 10 days of age, (3 animals for each group); 20 days of age - 2 animals; 1, 2, 4 and 6 to 7 pounds (3 animals for each group).

A separate series consisting of 14 fetuses from 4.5 to 12.5 cm., Crown-Rump length, and 61 kittens and cats was used for the chemical studies. (The age groups and numbers of animals used are presented in table 3 - page).

All adult cats and most of the kittens were obtained from the local pound. Some of the kittens were born in the animal quarters of this Department. The fetuses were delivered from the cats obtained from the pound.

B. Methods.

a) Histological Methods.

The histological methods of study included direct microscopic observations of stained preparations of the Suprasylvian gyrus as well as the quantitative determinations of the nuclear and cytoplasmic volumes of the neuron, or the neuron density and of the gray/cell coefficient. These were carried out on histological material from the departmental slide

collections which had been prepared in the following manner:

The animals were anesthetized and perfused with the physiological saline, containing 0.9 gm NaCl and 2.4 gm. gum acacia per 96.7 ml. water. The parts of skull covering the cerebral cortex were then removed, a larger block of cortex containing the suprasylvian gyrus was excized and placed for 24 hours in a fixative fluid compoased of: Bouin's fixative - 96.7 ml., NaCl - 0.9 gm., gum acacia - 2.4 gm.

The fixed tissues were then washed overnight with running water followed by many changes of 70% ethyl alcohol, until the fixative was removed. The suprasylvian gyrus was dissected out, dehydrated with graded ethyl alcohol solutions, cleared with xylol and embedded in paraffin.

The sections were cut at 25 μ in a direction perpendicular to the gyrus. Staining of the sections was carried out with buffered thionin at the pH 4.66.

The quantitative method employed for the histological studies was that of **CHALKLEY** ('43).

This method is based on obtaining a random distribution of points within the tissue under observation. This is accomplished by cementing four hairs (eye-lashes) to the eye piece of the microscope. Their four ends constitute the point-pattern. The preparation is moved at random under the microscope and the "hits", or structures (nucleus, cytoplasm, or "gray") on which these points fall are differentially counted by means of a blood counter.

In the present study the hits (nuclei and cytoplasm of the neurons) in the layers II through VI of the suprasylvian gyrus were counted in 10

sections of each sample, using every 5th section. The plane of the counts lay always at the depth of 15 μ below the surface of the section. The number of "hits" per section equalled 200.

The cell (neuron) density was determined by means of Chalkley's "circle" method ('49) using an eye piece equipped with a circle of a known diameter. This circle, when focused down into the tissue, "cuts out" a cylinder the volume of which can be easily calculated. In this study the circle was focused from 5 μ below the surface of the section to 20 μ below the surface. The diameter of the cylinder was 47 μ. The volume of the cylinder so delimited was 26,000 μ^3.

Only the nuclei of the cells recognizable as neurons were counted of which more than half of the nucleus lay within the cylinder.

All the cell counts were carried out at the magnification 1000. The microscope used was a Leitz monocular microscope equipped with a calibrated mechanical stage.

The calculation of the volume of the nucleus (or cytoplasm) was done by means of a following formula:

$$V_1 = \frac{V \times A}{100 \times D}$$

Where V is the volume of the cylinder, A - the mean % of the cytoplasm in the tissue D - mean neuron density (number of the nuclei) per cylinder.

The cell density was expressed as the number of nuclei per mm^3.

The gray/cell coefficient was calculated according to the definition of Haug ('50) by means of the following formula:

$$\text{Gray/cell} = \frac{V}{V_1}$$

where the meaning of the symbols is the same as in the first formula.

b) Chemical Methods.

The chemical methods were concerned with determination of the levels of the PNA, DNA and phosphoproteins expressed as phosphorus per gm. wet weight of tissue. The tissues for the chemical studies were obtained in the following way: The cat under study was anesthetized with nembutal, the skull over the gyrus opened (artificial respiration was maintained throughout the operation) and the animal plunged head-first (shoulder deep) into the liquid nitrogen. At this stage the artificial respiration was discontinued. After the cortex was frozen in vivo, a block containing the suprasylvian gyrus was chiseled out and placed on a block of dry ice on which it was immediately transferred to the cold room (at -10°C) where a small piece of the gyrus consisting of the entire thickness of the cortical gray matter was dissected out, weighed and ground with 5% TCA.

This procedure applied to all of the animals with the exception of the fetuses. In the latter case, the pregnant cat was anesthetized with nembutal and immersed chest-deep in warm saline. The fetuses were then delivered by caesarian section, and quickly plunged into liquid nitrogen. After the fetuses were frozen, the skull was opened and the rest of the procedure was carried out as described above. In all instances, all procedures involving ablation, freezing and dissection of the samples were carried out by Dr. Brizzee.

After the extraction of the acid soluble Phosphorus with ice-cold TCA and the phospholipids with ethyl alcohol, methyl alcohol chloroform mixture, and ether, the only Phosphorus compounds still contained in the solid phase were those of the PNA, DNA and the phosphoprotein fraction. The PNA was separated from DNA according to the method of Schmidt and

Tannhauser ('45). The phosphoproteins were separated from the PNA by precipitation with dry calcium hydroxide and the orthophosphate determined according to Allen's method.('40).

The phosphorus contents of the DNA and the phosphoprotein fractions were determined directly. The PNA phosphorus was calculated as the difference between the total phosphosphorus of the PNA + phosphoprotein fraction and the protein phosphorus.

The size of the samples did not always allow the separation of the phosphoproteins from the PNA phosphorus, therefore, in addition to the curve of PNA (fig. 3) an additional curve of the phosphorus of the PNA - phosphoprotein fraction is presented in fig. 4. It represents the trends of the PNA, since the phosphoproteins, although varying in amounts, do not show any particular trend, as will be seen later.

IV RESULTS OF THE STUDY

A. HISTOLOGICAL FINDINGS

The results of the qualitative morphological study (microscopic observations - see plates 1 and 2) are summarized in the table 1.

The results of the quantitative morphological study are presented in the table 1 and in the Fig. 1 and 2. The changes of mean volumes of the neurons were found to occur in the following pattern:

1. Nuclear volume.

The mean volume of the nucleus varied throughout the entire period under observation, being the lowest on the third day after birth ($377 \pm 50.3\ \mu^3$) and the highest ($635 \pm 93.5\ \mu^3$) in the 2 pound kittens.

2. Cytoplasmic volume.

The mean volume of the cytoplasm was $3.07 \pm 26.5\ \mu^3$ in kittens 1 day of age and remained unchanged on the third day of life. After this, it increased steadily until it reached its maximum of $991 \pm 226\ \mu^3$ in the 1 lb. kittens (about 40 days of age). The volume then decreased to $746 \pm 133\ \mu^3$ in 2 pound kittens subsequently leveling off in the 4 pound kittens ($754 \pm 91\ \mu^3$) and in the adult (6 - 7 lb.) cats - $725 \pm 65\ \mu^3$. The analysis of variance method of A. R. Fisher has shown the changes of the volume to be significant between the first and tenth day of age and highly significant from 20 days of age to one lb. kittens.

3. Cell volume.

The mean volume of the neuron was 718 ± 34 μ^3 in kittens one day of age. This value decreased (due to a decrease of the nuclear volume) to 681 ± 54 μ^3 in the kittens three days of age. After this, it increased steadily to a maximum of 1615 ± 262 μ^3 in the 1 lb. kittens. This was followed by a decrease to 1026 ± 149 μ^3 in the 2 lb. kittens. The lowest value (1176 ± 92 μ^3) was in the adult cats (6-7 lb.). The slight increase of the cellular volume to 1312 ± 171 μ^3 occurred in the 4 lb. kitten on account of an increase of the nuclear volume.

4. Nucleus/cytoplasm ratio.

The nucleus/cytoplasm ratio has been found to be 1.38±0.27 in kittens 1 day of age. From this time it decreased steadily to the 20th day after birth when the ratio was 0.60±0.02. After this the ratio fluctuated between 0.62 and 0.74.

5. The Cell density.

The changes of the cell density are reported in the table 1 and Fig. 2. The mean cell density decreased steadily from 1.68 x 10^5 cell/mm^3 in the kittens 1 day of age to 0.46 x 10^5 cell/mm^3 in the 1 lb. kittens In the 2 lb. kittens the density increased to 0.56 x 10^5 cell/mm^3 then it decreased again to 0.46 x 10^5 cell/mm^3 In the adult cats the cell density was 0.44 x 10^5 cell/mm^3.

6. Gray/cell Coefficient.

The mean values of the gray/cell coefficient increased continuously from 8.0 ± 1.01 in the kittens 1 day of age to 10.6 ± 75 in the kittens 10 days of age. In the kittens 20 days of age the value of the gray/cell coefficient was still 10.6 ± 60. After this the values continue to increase steadily to 19.5 ± 2.18 in the adult cats.

TABLE I

Morphological changes in the suprasylvian gyrus of the cerebral cortex of the cat.

Stage of Development	Changes in the cortical zone.
4.5 cm fetus	The zone is not subdivided into layers. The cells larger than those of the ependymal zone, have vesicular nuclei, scanty cytoplasm. The cells are arranged in cords.
12 - 12.5 c. fetus	The 5th (pyramidal) layer can be distinguished. Cord-like arrangement of the cells is interrupted in the 5th layer. Large nuclei of the future pyramidal cells are well discernable. There are also a few young pyramidal cells. 6th (multiform) layer incomplete. Unipolar and some bipolar neuroblasts in the 5th and 6th layers and a few spongioblasts. The upper part of the zone still not subdivided. Cells very immature, arranged in cords.
1 day of age kitten	More "young" pyramidal cells in the 5th layer. More spongioblasts and some neuroglia can be distinguished in 5th and 6th layers. The rest of the zone still not subdivided, the part of the zone just beneath the nolecular layer is very dense. Cells immature, arranged in cords.
kittens 5 days of age	Better development of the 5th and 6th layers. Pyramidal cells of the 5th layer are plainly recognizable. No subdivisions in the outer part of the zones. The cells in it are larger, many neuroblasts and some spongioblasts, a few neuroglia are present. The cells are still arranged in cords.
kittens 10 days of age	The 2nd (granular), 3rd (pyramidal) 4th (granular) layers are distinguishable. Better development of the 5th and 6th layers. 2nd layer contains a great number of indifferent cells, but maturer forms also present. Pyramidal cells are seen in the 3rd layer. Small neuroglia recognizable in all layers. Bundles of fibers are visible.
kittens 20 days of age	Many new neurons in the upper layer, but the embryonic character of the 2nd (granular) layer is not yet lost. The layers still contains many indifferent cells. The rest of the layers well developed. The cord-like arrangement of cells is lost.
1 pound kittens	Better development of 2nd layer but the indifferent cells still persist in its outermost part.
2 pound kittens	Many new neurons in upper layer, particularly in the 2nd (granular) layer. Good development of all layers.

TABLE 2

Quantitative Morphological Changes in the Cerebrae Cortex during postnatal development.

Group of Animals	Age	Mean Weight	No. of Animals	Volume of the neuron μ^3 Nucleus	Cytoplasm	Cell	Ratio Nucl./Cyt.	Neuron Density (x 10^5) mm^3	Gray/cell Coefficient
Kittens	1 day	103 gm	3	416±46	304±26	718±34	1.38±0.27	1.68±0.14	8.0±1.01
	3 days	141 "	3	377±50	304±30	681±54	1.25±0.21	1.54±0.12	9.5±1.36
	5 days	164 "	3	393±19	456±28	850±71	0.88±0.16	1.18±0.11	10.2±1.57
	10 days	210 "	3	513±183	638±185	1150±400	0.80±0.16	0.91±0.26	10.6±0.75
	20 days	227 "	2	557±86	956±169	1530±252	0.60±0.02	0.63±0.06	10.6±0.60
		1 lb.	3	635±93	991±226	1615±262	0.66±0.19	0.46±0.04	13.6±0.95
		2 lbs.	3	460±72	746±132	1206±149	0.63±0.17	0.56±0.05	14.9±1.72
		4 "	3	558±89	754±91	1312±171	0.74±0.07	0.46±0.04	16.9±3.31
Cats		6-7 "	3	425±39	725±65	1176±92	0.62±0.05	0.44±0.01	19.5±2.18

B. CHEMICAL CHANGES

The results of chemical determinations are presented in table 3, and Figures 3 and 4.

Phosphoproteins:

Some of the spectrophotometric reading for the phosphoproteins were too low to be accurately evaluated. However, in the instances where it could be done with a reasonable degree of accuracy the phosphoproteins did not show any significant changes, the value being 153 γ/gm in 4.5 cm fatuses, 126 ± 11 γ/gm in 12.5 cm fatuses, 125 ± 17 γ/gm for 1 day old kittens and for the adult cat the average values were: 118 ± 16 and 135 ± 33 γ/gm for 5-7 lb., and 7-10 lb. groups correspondingly.

The method of the variance analysis (Fisher - '54) confirms the statement that there is no significant difference in content of phosphoproteins between the different age groups.

PNA

The average values for the PNA after separation of phosphoproteins were: 290 γ/gm in 4.5 fetuses, 168 ± 12 γ/gm in 12.5 cm fetuses and 201 ± 9 γ/gm and 167 ± 38 γ/gm for the two groups of adult cats. (In the guinea pig, Flexner and Flexner (10) found the range for PNA to 230 - 270 γ/gm on the 30th day of gestation and 80 γ to 290 γ/gm in the adult guinea pig.

The analysis of variance method of Fisher ('54) failed to show any significant differences in the PNA content in the age groups from 1 day of age to 335 gm, and from 335 gm to 10 lbs., but the decrease of PNA phosphorus during the fetal period proved to be significant.

DNA

Desoxypentose nucleic acid levels decreased steadily with only slight fluctuations in rate from an average level of 432 ± 22 γ/gm in 4.5 cm fetuses to 62 ± 7 γ/gm in 11 day old kittens. In older kittens and adult cats the levels were more or less constant varying between 59 and 73 γ/gm.

The decrease of the phosphorus level of DNA was highly significant during the fetal period, and significant for the group from 1 day of age to 11 days of age. There were no significant changes in the animals from 11 days of age to 10 lbs.

PNA/DNA Ratio

The ratio PNA/DNA was quite low in the youngest fetuses used (0.66). It rose to 2.04 in the kittens 3 days of age, then decreases again to 1.29 on the 5th day after birth. In the later stages of growth and in the adult cats it fluctuates between 2.71 and 3.67.

TABLE 3

Phosphorus Levels of the PNA + Phosphoproteins, PNA, Phosphoproteins and DNA Fractions

Animals	Length cm	Age	Mean Weight	No. of Animals	Content of Phosphorus γ/gm of tissue				Ratio
					PNA + ph.prot.	Phospho. Prot.	PNA	DNA	PNA DNA
Cat fet.	4.5			3	443±17	135*	290*	432±22	0.55
"	10.5			5	306±44			203±20	
"	12.5			6	277±25	126±11***	168±12***	173±11	1.00
Kittens		1 day	80 gm	3	330±20	125±17	205±12	135±6	1.52
"		2 days	81 "	4	298±11			118±16	
"		3 "	119 "	6	333±47	150*	233*	126±15	2.04
"		4 "	135 "	3	359±11			105±14	
"		5 "	133 "	2	335±30			106±6	
"		8-10 "	162 "	5	313±8	120*	194*	83±6	1.29
"		11 "	146 "	2	252±6			62±7	
"		26 "	205 "	2	280±5			59±0	
"			335 "	5	303±22	129±10	174±20	65±9	2.72
Older Kittens			1-1.99 lb.	6	298±25	145±1**	182±6**	58±7	3.62
"			2-2.99 "	4	282±28	119*	204*	59±13	
"			3-3.99 "	4	299±46	127*	131*	66±24	2.94
"			4-4.99 "	1	288	109	179	59	
Adult Cats			5-5.99 lb.	5	321±9			57±15	
"			6-6.99 "	5	347±42	118±16	201±9	58±6	3.58
"			7-10 "	4	302±56	135±33	167±38	64±5	2.71

* 1 determination
** 2 determinations
*** 3 determinations

V. DISCUSSION

For different species of animals there are differences in the relative time of growth of the central nervous system as well as in the degree of its differentiation at the time of birth. Tilney states that the guinea pig has a higher degree of development of the cerebral cortex at the time of birth than the cat does. On the other hand, the central nervous system of a new born cat is much better differentiated than that of the new born cat.

An accumulation of quantitative data for the stages of the normal development of all experimental animals are extremely important as the basis for experimental research. Such data are completely lacking for the cat.

The cat is one of the most important experimental animals, used in many branches of biological research. Therefore, the selection of the cat as an animal of choice for this study presents an attempt to fill in this "gap".

As it has been stated previously, the cats used were of both sexes and of all sizes and of mixed or mongrel breeds, and in most cases of a completely unknown background. This presented a certain handicap in establishing a common frame of reference such as age against which the histological and chemical data could be plotted. It has been decided to use age as frame of reference where it was definitely known. In other cases it was necessary to use weights. Although the weight can be influenced by factors other than growth (state of nutrition, breed and other) in the great majority of cases it proved to be a satisfactory criterion of developmental stage.

Since the fetuses were delivered from the cats already pregnant at the time when they were obtained from the dog pound, their exact age was unknown. The age grouping of the fetuses was, therefore, performed on the basis of the crown-rump length expressed in centimeters.

The criteria used for the determination of the degree of development were: 1) the size of the cell and its parts (nucleus and cytoplasm); 2) the ratio nucleus/cytoplasm; 3) the neuron density; 4) the gray/cell coefficient; 5) the level of nucleic acids and their ratio; 6) the degree of differentiation of the cortical layers and cells as observed by direct microscope examination. The criteria of growth employed by a student of development or maturation depend on what one understands by the term "development". Thorell ('47) gave the definition of the phenomena of growth and development in the following words: "The fundamental process of development . . . involves growth by increase in mass and differentiation into functionally specialized tissue cells. The cellular growth can be defined, whether it takes place by division or by enlargement of the cell, as the formation of the fundamental substances of the cell. From the quantitative point of view the latter consists essentially of the protein substances of the cell". This statement can be equally well applied not only to the cell, but to the whole structures.

Nissl (1898) observed that the cell density is lowest in the animal species with the highes organization of the central nervous system. The phylogenetic studies of von Bonin ('33-'39), and Haug ('53) and others, confirmed this observation. Brody's ('55) studies of human cortices of different age groups from infants to 95 years old, have shown that a

similar relationship existed between the cell density and the degree of organization of central nervous system at different stages of development and maturation. Thus the cell density at a given stage can be used as a criterion of the degree of development. In the present studies a steady decrease of neuron density was observed in kittens from 1 day of age to 1 pound.

While the neuron density means the number of cells per/mm^3 of the cortical substance, the gray/cell coefficient expresses the relationship between the volume of the gray substance and the total cellular volume. It is, therefore, a good criterion of the relative growth of the gray substance versus the growth of cell bodies. The use of the cellular size as a criterion of growth is obvious and does not require further explanations. The ratio nucleus/cytoplasm is a criterion of the differentiation of the neurons.

Finally, the levels of the nucleic acids were used as criteria of development of the cerebral cortex because of their commonly recognized role in the development of the cell.

It has been stated previously that paraffin sections were used for the quantitative morphological studies. In order to avoid errors caused by the changes of volume due to the shrinkage or swelling of the brain tissue in the process of tissue preparation, gum acacia was added to the perfusing and fixing fluids (Koenig, Groat and Windle '45). Gum acacia prevents swelling of the tissues by creating in the blood vessels osmotic pressure which is high enough to oppose the hydrostatic pressure in the capillaries.

The method of Chalkley was used for the quantitative morphological analysis. The main reason for the selection of this particular method was its objectivity and universality. While all the other methods including those of von Economo ('25) von Bonin ('37) Agduhr ('41), Haug ('53, '53) involve direct or indirect measurements of the cells and calculate their volumes on the basis of their relative similarity to different geometrical figures, Chalkley's method is completely independent of the shapes of the cells. The main source of errors is, thus eliminated. This is particularly important in the developmental studies considering the multiformity and changeability of the shapes of cells during growth.

Another advantage of Chalkley's method is its relative simplicity. Shariff ('53) characterized this method as an "elegant" method, and Haug ('57) recognized its objectivity.

Inspite of the objectivity of this method, extreme care should be exercised by the investigator using it in evaluation of the position occupied by the cell in the space in which the cells are counted. In this study a neuron was counted only in cases where more than half of its nucleus lay within the cylinder. Only "direct" hits of the nucleus through its central pointer and the "hits" of the cytoplasm in which the pointer was or a well defined portion of cytoplasm were counted. (If "shadows" of cytoplasm or nucleus are also counted, the volume of the cell and the cell density will be artificially increased.)

The chemical method chosen for the present study involved preparation of the brain tissues for the determination of the phosphorus content of the DNA, PNA and phosphoprotein fractions after removal of the acid soluble

phosphorus and the phospholipids. The artificial respiration was maintained during all the time the animal was on the operating table up to the moment when its head was immersed into liquid nitrogen. This was done in order to eliminate possible hyposig.

The brains were frozen in vivo with liquid nitrogen and kept in the frozen state during dissection and weighing. Freezing prevent loss of weight of the samples due to evaporation of water contained in the tissue fluids, as well as the hydrolysis of the nucleic acids.

Of the quantitative methods for the determination of the nucleic acids those most suited for our purpose were the method of Schneider ('46) based on the pentose determination, and the method of Schmidt and Thannhauser ('45), based on the phosphorus determination.

The disadvantage of the methods based on determination of pentose is the fact that some other substances like glycoproteins, which may be present in the tissue, interfere with the color reactions for the pentoses.

The modified method of Schmidt and Thannhauser ('45) was selected because this method is based on Phosphorus determination and makes possible a comparison of the relative contents of all the phosphorus compounds in the sample (the other findings are not reported at the present time, being a part of another project). The major modifications of the method of Schmidt and Thannhauser included a direct determination of the content of DNA, and precipitation of the phosphoprotein with powdered calcium hydroxide rather than with a suspension of magneusium carbonate in a calcium chloride solution as used in Delory's method. Delory's ('38) method was tried first, but proved to be inaccurate in the studies with

small amounts of phosphorus. (It did not work even with the standards in the range from 1 - 10 phosphorus).

R. J. Allen's ('40) method for the colorimetric determination of ortho phosphate was selected for the determination of the phosphorus levels.

The advantages of the method are: the ease and completeness of digestion of the tissues with perchloric acid, the fast development of color (5 minutes), the intensity of which does not change for about 30 minutes.

The colorimetric determinations were carried out in the Bausch and Lomb Spectronic 20 colorimeter at 600 mμ.

Tilney states that the cortex of the 120 mm cat is divided into six layers: marginal, 1st granular, pyramidal, 2nd granular, mantle, ependymal.

It is the opinion of the author that this division is somewhat misleading. It appears preferable to use the term "zone" when applied to the fetal tissues. When the word "layer" is used, one thinks of it in the sense in which it is used for the adult cortex: I, (molecular- corresponds to the marginal zone of the embryonic stages); II, (external granular); III, (external pyramidal); IV, (internal granular); V, (internal pyramidal) and VI (multiform).

It would be better to say that in 120 mm cat the cortex is composed of 4 zones: (marginal, cortical, mantle and ependymal) of which one - the cortical zone - is divided into 3 layers: a wide layer (approximately

one-half of the cortical zone); a layer containing large nuclei with scanty cytoplasm, and a few better differentiated pyramidal cells, (V layer of the adult cortex) and a layer containing small and slightly larger undifferentiated (indifferent) cells, (the VI or multiform layer of the adult cortex).

The comparison of the data presented in table No. 1 of this thesis with the data obtained by Peters and Flexner ('50) in their studies of the developmental stages of the guinea pig shows that the cortex of the guinea pig is much more developed at birth than that of the cat (table 4).

TABLE 4

Relative times of development of cerebral cortex in cat and guinea pig.

Criteria of Maturity (Used by Peters and Flexner)	Cat	Guinea Pig
1. Time of the final differentiation of the cortical layers	10 days of age	31 days of gestation
2. Loss of cordlike arrangement of the cells	1st signs appear in 12.5mm fetus	1st signs 41st day of gestation
3. Definitive* pyramidal cells in the 5th layer	3-5 days of age (but a few young pyramidal cells can be seen in some 12.5mm fetuses)	53 days of gestation

*This is an expression used by Peters and Flexner. Under definitive "pyramidal cells" I understand those which have a definite shape of a pyramidal cell.

The quantitative morphological studies were carried out in an entirely different series of the animals than those used for the chemical determinations and for the qualitative studies of morphological changes in the fetal stages. Since no fetuses were available at the time when the quantitative morphological studies were carried out, and since the fetal tissues were fixed only with formalin (which could cause differences in the volumes as compared with the tissues in the 2nd series), on the postnatal morphological changes were studied quantitatively.

The mean volume of the neurons was 718 μ^3 in the kittens one day of age, and 1176 μ^3 in the 6 - 7 pound cats. This shows the overall growth of the cell during the period of postnatal development. The increase in size occurs on the account of the cytoplasm (304 μ^3 in the kittens one day of age to 725 μ^3 in the adult). The size of the nucleus does not change appreciably (416 μ^3 in the kittens one day of age and 425 μ^3 in the adult). The changes of the cellular volumes go through the following stages (fig. 1): Stage 1 - from one to three days of age there is practically no change in the volume of the cells; Stage 2 - from three days of age to 1 pound kitten the cellular volume increases due to increase of both nucleus and cytoplasm. Stage 3 - from 1 pound to 2 pounds the volume of the cell decreases rapidly. Stage 4 - from 2 pounds to the adult. During this period there is little change in the cytoplasmic volume. The values for the nuclear volumes increase slightly in the 4 pound cat and decrease again in the adult. The corresponding values for the cellular volumes are 1312 μ^3 and 1176 μ^3. These data for the adults are in good agreement with the data of Shariff, who found the mean volume of the neuron for the chimpanzee

to be 1035 μ^3, and the data of Peters and Flexner, who reported the mean volume of the neuron in the guinea pig to be 1450 μ^3.

The changes in the nucleus/cytoplasm ratio are presented in the Table 1 and figure 1. The n/c ratio decreases during the period from the kittens one day of age to the one pound kittens. The decrease is due to the increase in the size of the nucleus which attains its maximal volume (635 μ^3) at this stage. The ratio decreases further in the two pound kittens. At this stage there is a decrease of both - the nuclear volume and cytoplasmic volume, but this change is more significant in the cytoplasm. In the 4 pound kittens the ratio increases due to the temporary increase of the mean nuclear volume. In the adult cat the n/c ratio decreases again.

The increase of the mean cellular volume of the neuron during the period from three days of age to one pound kittens is followed by a sharp decrease between the one pound and the 2 pound stages. These phenomena can be caused by any or all of the following reasons: a) the increase of the volume could be due to errors in calculation: Since the upper layers of the cortical zone are poorly developed during the early postnatal period, the cell counts for the stages from one day of age to three days of age may have included some spongioblasts besides the neurons and neuroblasts (especially in the upper layers). After differentiation into recognizable neuroglia during the subsequent stages they were bound to be excluded from the counts. This would cause a decrease in the cell density and an increase of the mean volume. The error is not likely to be very significant, but repeated differential counts of cells in these layers should be carried out

in the future to prove it. b) The increase may be due to the true growth of neurons which have been differentiated before the 3 days of age stage, and finally to c), an increase of the volume of the cells due to the increased functional activity. It is known (Weiss-1955 and other authors) that this process occurs in functionally overloaded cells. It involves both the nucleus and cytoplasm of the cell. After the load diminishes, the volume during this period could occur if the interstitial tissues of the cortex of young kittens were less susceptible to shrinkage than those of older animals.

The decrease of the mean cellular volume in the 2 pound kittens could be caused by a), an increase in density due to the appearance at this particular time of many new neurons of smaller size. (A slight increase of the density actually occurs at the 2 pound stage.) b). By a shrinkage or involution of cells after the decrease of the activity of the cells if the preceeding increase in the volume was due to "overloading" of the cells by functional activity (protein synthesis growth of the cytoplasmic process or some other kind of activity.

Examining the curves for the mean nuclear, cytoplasmic and cellular volumes (Fig. 1 - 2), one can see that the curves for the mean nuclear, cytoplasmic and cellular volumes (Fig. 3) show fairly high standard deviations which could be partly due to the fact that the relatively small number of animals used for this study was not sufficient to obtain statistically correct standard deviations. However, there is another explanation: it appears that the morphological changes are determined by two factors - age and body weight rather than by the age alone. The

relationship between the age, body weight and morphological changes is shown in table 5.

TABLE 5

The relationship between age, body weight and the morphological findings.

Serial No.	Age	Weight gms.	Volume μ^3			Density
			Nucl.	Cytopl.	Cell	
B 63	3 days	147	433	309	742	1.61
B 56	5 days	147	384	285	669	1.57
B 50	5 days	153	378	544	922	1.30
B 49	unknown	422	542	1126	1668	0.46
B 54	unknown	478	630	730	1330	0.50

These data show that the rate of growth is not the same in all animals and therefore at those periods at which the greates changes occur, differences are to be expected and the standard deviations will be higher than in adult animals.

As has been pointed out before, the cell density is an important criterion of the degree of organization of the central nervous system in phylogenetic studies. The present study shows that the density can also be applied as a criterion of the degree of maturity during the developmental period. The studies of Brazzee '57-'58* show the same general trends of the cell density changes for a different region of the cat brain during the

*Unpublished data

period of growth.

The changes of the cell density of the cerebral cortex occuring during the period of postnatal development are shown in the table 1 and fig. 2. The mean cell density decreased rapidly during the first 10 days. After this the rate of decrease diminished. At this stage the cell density did not differ appreciably from the cell density of the adult cat and no further changes occured during the period under observation, except for a slight elevration in the 2 pound kittens. The density for the adult cats was 0.44×10^5 c/mm^3. The density reported for the adult cat by Tower ('54) and Heller and Elliott ('54) was 30,000 c/mm^3 and 90,000 c/mg respectively. (Heller and Elliott used a hemacytometer to count the nuclei suspended in cold citric acid solution and stained with polychrome methylene blue.) The decrease of the cell density in the kittens from 10 days of age to one pound shows that during this period the rate of growth of the interstitial substance is higher than the rate of growth of the neuron perikryou during the same period. The elevation of the density in the two pound kittens may be a reflection of the simultaneous differentiation of a considerable number of new neurons, namely in the upper cortical layers of the cortex. This phenominon was observed at this stage during the course of qualitative morphological studies. Since the cell density does not change appreciably after the four pound stage, it appears that the differentiation of the neurons is essentially completed by this time.

The curve of the gray/cell coefficient (table 1, Fig. 2) shows a steady increase of the g/c coefficient during the period from birth to the adult stage with the exception of the period from 10 to 20 days of age when its level is constant. The values for the g/c coefficient were 8.0 for kittens one day of age and 19.5 for the adult cat.

Generally the increase of the g/c coefficient during the process of growth and maturation reflects a continuous differentiation of the brain cortex.

There are two factors affecting the g/c coefficient: the cell density and the mean volume of the neuron.

The increase of either would bring about the decrease of the g/c coefficient. Therefore, the relationship between the gray/cell coefficient and the density, as well as between the g/c and the total cell volume is a reversed one.

From this standpoint two of the developmental stages are of particular interest: the stage from 10 to 20 days of age and the stage from 4 pounds to the adult cat.

During the first stage the g/c coefficient remains constant, although the density goes down. This period coincides with an increase of the cellular volume. During the second stage (from 4-6 pounds) the g/c coefficient continues to increase while the cell density shows little change. This increase of the g/c coefficient is due to the decrease of the total cellular volume. Whether it means a differential shrinkage of the cells in adult cats or the involution of the cells can, be shown only by further studies. If true shrinkage of the cells will be confirmed it would seem that the increase of the g/c coefficient, after the differentiation of the cells is essentially completed, indicates a "regression" of the cellular population rather than a further differentiation of the cerebral cortex.

The average value of the g/c coefficient was 19.5 for the adult cat in this study, which is well in the range of the g/c coefficient found by Shariff (g/c = 20) for the chimpanzee and with Haug's value of 35 for the sheep.

The changes of the levels of the nucleic acids were the main object of the chemical part of the present study. As has been pointed out before, the size of the samples did not allow the separation of the PNA from the phosphoproteins in all the tissues studied. On the other hand, the data concerning phosphorus levels in the combined PNA phosphoprotein fraction were available for all animals studied. The study of the phosphoprotein levels (when possible) was therefore indicated in order to ascertain how their presence affects the total phosphorus levels of the combined PNA - Phosphoprotein fraction. Since this study failed to reveal any definite trend of changes of the phosphoprotein fraction, the curve for the phosphoprotein+PNA (Fig.4) can be regarded as reflecting the trends of changes of the PNA.

The decrease of the mean content of the PNA phosphorus (PNAP) from 290 γ/gm in the s4.5 cm. fetuses to 168 γ/gm in the 12.5 cm fetuses confirms the findings of many authors, including Flexner and Flexner, ('51) Thorel ('47), Davidson ('47) etc., that the levels of the nucleic acids, high in the earlier stages of the gestation period, decreases gradually with progressing growth. Flexner and Flexner found a decrease of the PNAP level for 279 γ/gm on the 28th day of gestation of the guinea pig fetuses to 133-210 γ/gm on the 66th day of gestion. The content of the PNAP reported by these authors in the cerebral cortex was 91 - 299 γ/gm.

In the present study, the mean values for the PNAP fluctuated between 170 /gm to 200 /gm in the kittens and cats from the first day of life to the adult stage. The PNAP changes during the period of

the prenatal development proved to be significant by the method of variance analysis of R. A. Fisher ('54).

The only instances in which the mean values for the PNAP contents deviated appreciably from this more or less constant level were in one kitten 3 days of age (233 γ/gm) and in one 4 pound kitten (131 γ/gm).

These data seem to indicate that the level of the PNAP becomes stabilized shortly before birth.

The mean level of the DNAP, unlike that of the PNAP, does not stabilize until the 11th day after birth. In the present study the content of the DNAP decreased from 432 γ/gm in the 4.5 cm. fetuses to 62 γ/gm in the 11 day old kittens. After this time the mean values for the DNAP fluctuated from between 59 γ/gm throughout life. The highest individual phosphorus content for the adult cat was 97 γ/gm, the lowest 43 γ/gm.

These data are in good agreement with the data of Flexner and Flexner ('51) who reported the DNAP content in the adult guinea pig cortex in the range from 30 γ/gm to 100 γ/gm.

Heller and Elliott ('54) found the content of DNA in the cerebral cortex in the range of 63 γ/mgm to 105 γ/gmg or the recalculated DNAP=55 γ/gm to 92 γ/gm.

It should be noted here that the chemical changes in the cortex, like the morphological changes, are determined by both the age and the weight of the animals. This can be illustrated by the following example: 3 kittens from the same litter all 2 days of age but with different weights,

show the following content of the DNAP: the 75 gm kitten - 136 γ/gm; the 80 gm kitten - 117 γ/gm; the 89 gm kitten - 97 γ/gm.

The ratio PNA/DNA increased steadily from 0166 in the 4.5 cm fetus to 2.04 in the kittens 3 days of age. It decreased then to 1.29 in the kittens 8 - 10 days of age. This decrease was followed by a new increase of the ratio to 3.62 in the 1 - 2 pound kittens. In the 3 - 4 pound kittens the ratio decreases again to 2.94, only to increase to 3.58 in the adult cats. In the 7 - 10 pound cats the ratio goes down again to 2.71.

These data agree very well with those reported by Flexner and Flexner ('51) for the guinea pigs. The ratio PNA/DNA from their table no. 1 is 0.58 on the 32nd day of gestation. The ratio for adult animals calculated from their fig. 1 is about 3.55.

The stabilization of DNA level occurs on the eleventh day after birth. At about the same time the mean volume of the perikaryon attains the value equal to that of the perikaryon of the adult cat. Both observations suggest that the process of "true" growth of the perikaryon is essentially completed on the 10 - 11th day after birth. The period from 10 to 20 days after birth is characterized by a further increase of the mean cellular volume accompanied by a constant level of the gray/cell coefficient and by a decrease of the cell density. While the decrease of the cell density indicates a further growth of the gray substance, the increase of the mean cellular volume is only temporary and suggestive of a swelling of the cell due to increased functional activity of the

perikaryon and rather than of the true growth of the cell body. On the other hand, the constancy of the level of the gray/cell coefficient indicates that the rates of growth of the gray substance and of the cellular swelling are equal during the period from 10 to 20 days after birth.

Therefore it seems logical to assume that both rates are functions of a common factor, which is likely to be the functional activity. In other words, the relationship between the mean cellular volume and the volume of the gray substance suggests that the growth of the gray substance during the period from 10 - 20 days after birth is due to the growth of some structures formed by the perikaryon. There is a good possibility that these structures include the cytoplasmic processes of the neuron and the myelin sheaths. The slowing down of this process during the period from 20 days of age to 1 pound kittens is suggested by a decrease of the rate of cellular swelling and by an increase of the gray/cell coefficient. Between the 1 pound and 2 pound stages cellular involution sets in, as suggested by a sharp decrease of the mean cellular volume, which goes down to its normal value in the 2 pound kittens. It appears that the development of the cerebral cortex is complete in the 2 pound kittens since no further appreciable morphological changes occur after this time.

It should be kept in mind however, that the succession of events outlined here is only hypothetical and has to be proven by further research involving the phase microscopy and elecrophysiological studies.

It is the opinion of the author that the following studies of the problems arizing from the present studies are also desirable: 1) studies of the susceptibility to shrinkage of the cortex in cats of different ages. 2) studies of the relative numbers of glia and neurons in different layers of the cortex of the cat and the changes of the glia/neuron index during development. 3) studies of morphological and chemical changes in the elements other than the cell (fibers, myelin sheets) of the cerebral cortex of cat during the period of development. 4) studies of chemical and morphological changes in the aging cerebral cortex of cat.

VI SUMMARY

101 animals were used for the study of morphological and chemical changes in the suprasylvian gyrus of the cat. The prenatal studies were carried out in the fetuses for 4.5 cm to 12.5 cm. C-R length. The postnatal changes were studied in the cats from 1 day of age kittens to the adult stage. The quantitative morphological studies included studies of changes of the mean cellular volume of the nucleus/cytoplasm ratio, the density of the neuron and of the gray/cell coefficient. The quantitative methods employed were those of Chalkley ('43 and '49). The qualitative morphological studies were carried out by means of microscopic examination of histological preparations. The chemical studies were concerned with determination of the content of the nucleic acids and phosphoproteins. The methods employed were those of Schmidt and Thannhauser ('45) and Allen ('40).

A hypothetical succession of events taking place during the postnatal period of development of the cerebral cortex of the cat was outlined.

The data obtained during the studies indicate that the morphological and chemical changes are determined by the age and body weight of the animals.

The overall quantitative morphological changes during development were a decrease of neuron density, and of the nuclear/cytoplasmic ratio an increase of the mean cellular volume, of the nucleus/cytoplasm ratio and of the gray/cell coefficient. The qualitative morphological studies give evidence of the stage-by-stage occurring differentiation of the cortical layers and of the cells in the layers. The data of the chemical studies seem to indicate that the content of the phosphoproteins per gm of wet tissue is maintained at more or less constant level during the entire period of development and throughout the adult life. The content of PNA decreased during the fetal life but did not change significantly from the 1st day after birth to the adult stages, while the content of the DNA decreased during the prenatal period and after birth, and became stabilized only on the 11th day after birth.

Further morphological and chemical studies developing and aging cerebral cortex of the cat are suggested.

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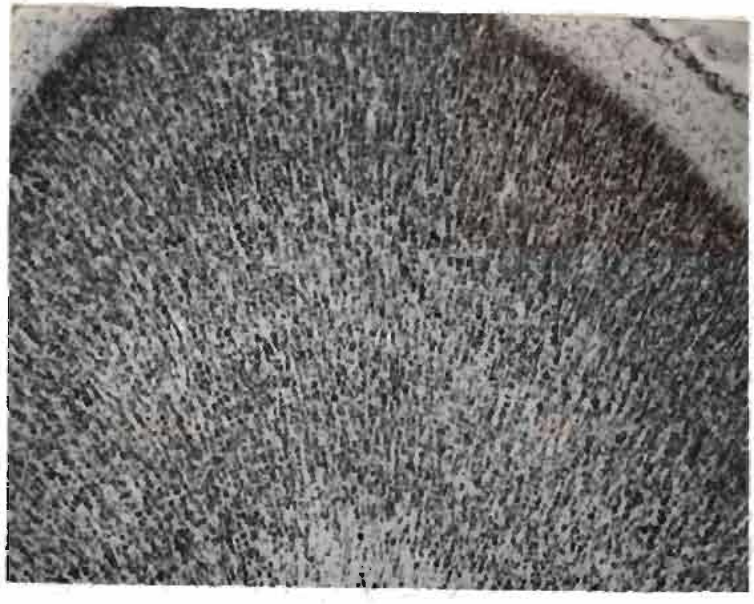
PLATE 1

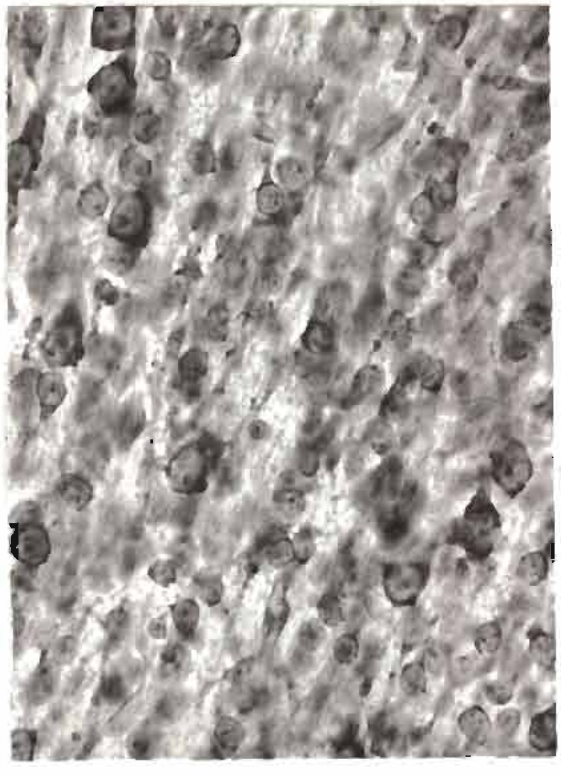
Suprasylvian gyrus of the Cerebral Cortex of the 1 day old kitten.

A. Low power view

B. High (dry) power view of cells of the layers V and VI



A



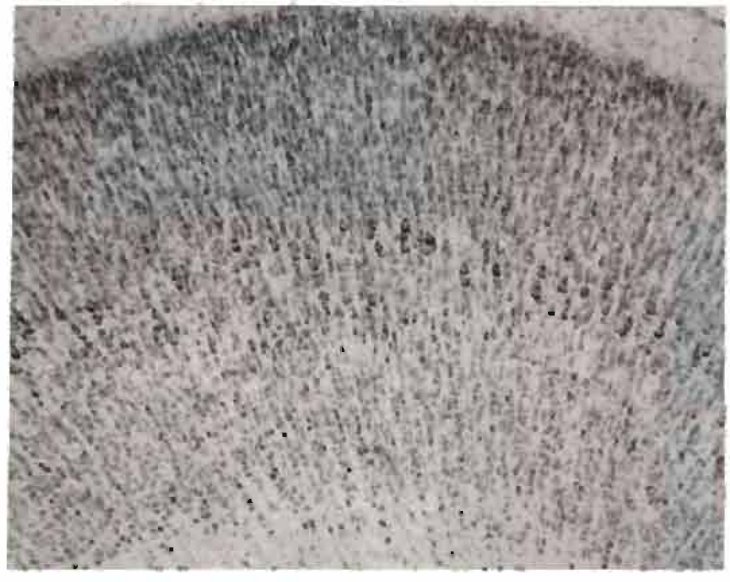
B

PLATE 2

Suprasylvian gyrus of the Cerbral Cortex of 10 days old kitten.

A. Low power view

B. High (dry) power view of cells of layers II and III



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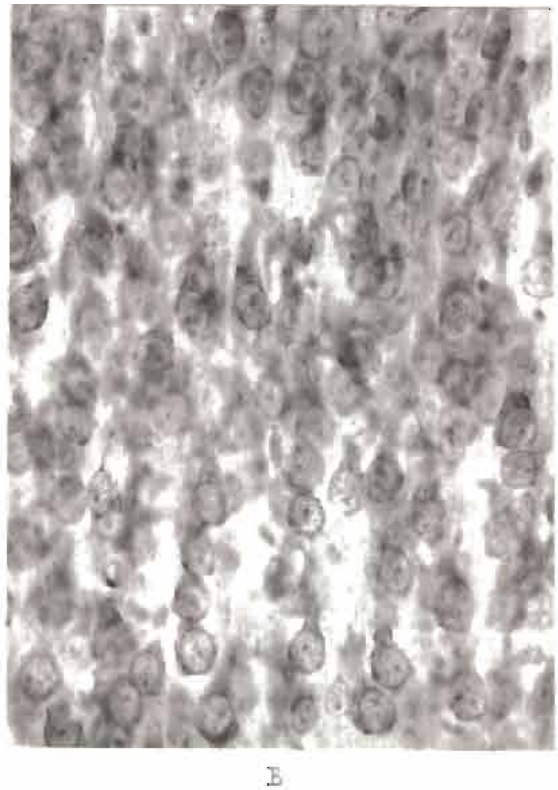


B

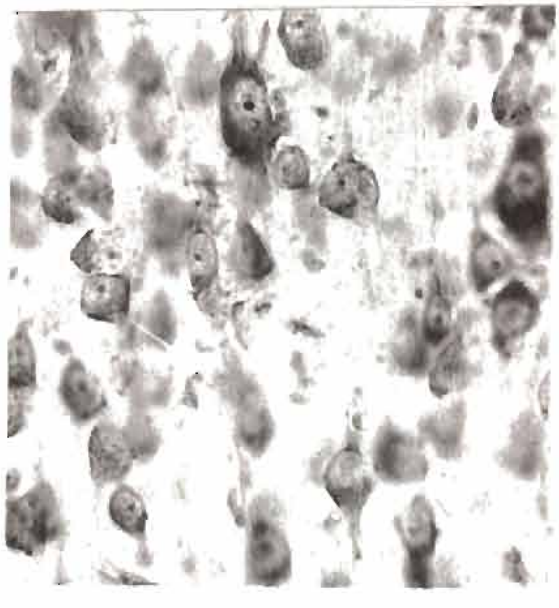
PLATE 3

Suprasylvian gyrus of the Cerebral Cortex of 1 pound kitten

A. Low power view

B. High (dry) power view of pyramidal cells of the layer V

A



B

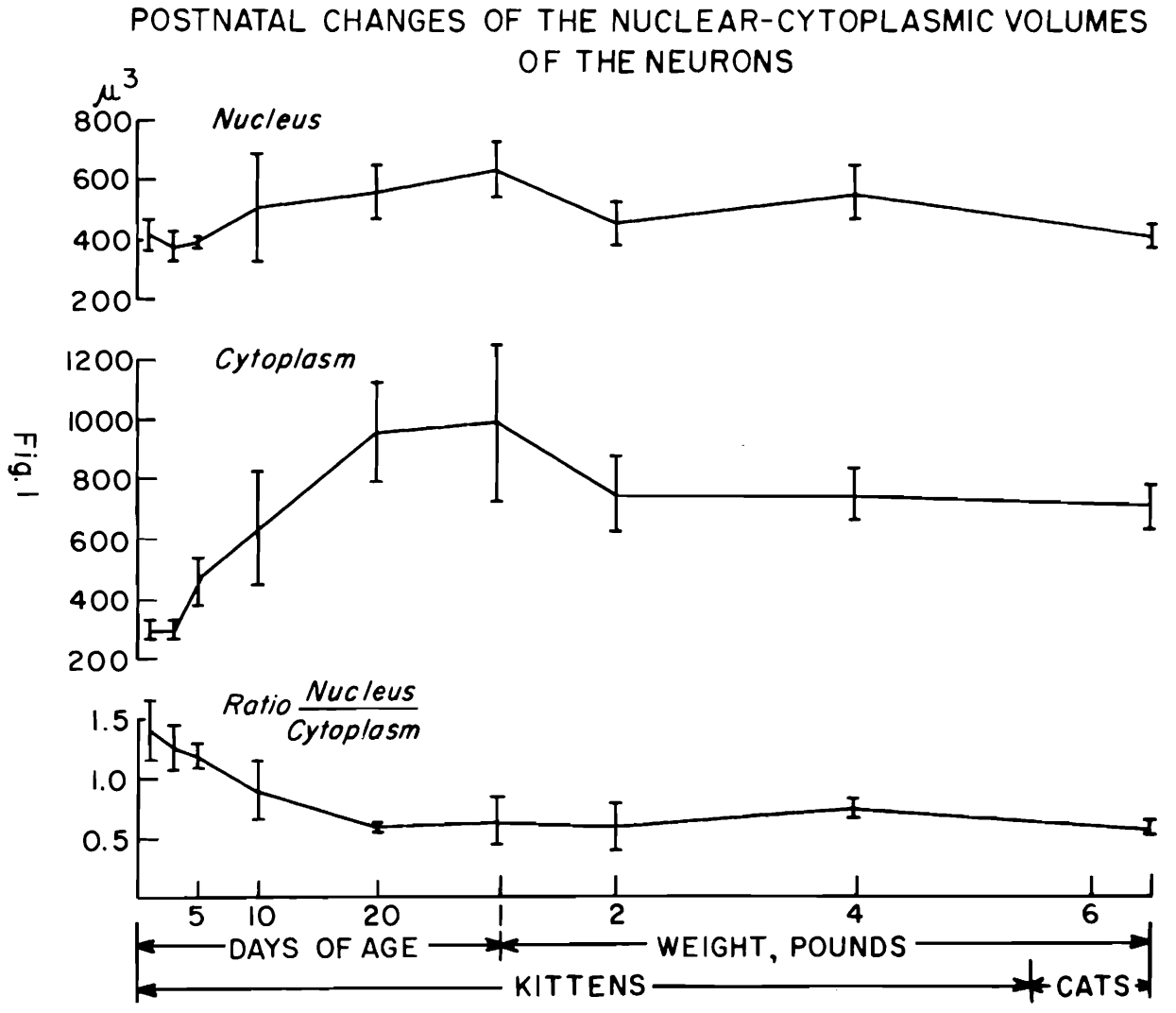

POSTNATAL CHANGES OF THE NUCLEAR-CYTOPLASMIC VOLUMES OF THE NEURONS
μ3
Nucleus
800
600
400
200
Cytoplasm
1200
1000
800
600
400
200
Ratio Nucleus / Cytoplasm
1.5
1.0
0.5
5
10
20
1
2
4
6
DAYS OF AGE
WEIGHT, POUNDS
KITTENS
CATS

Fig. 1

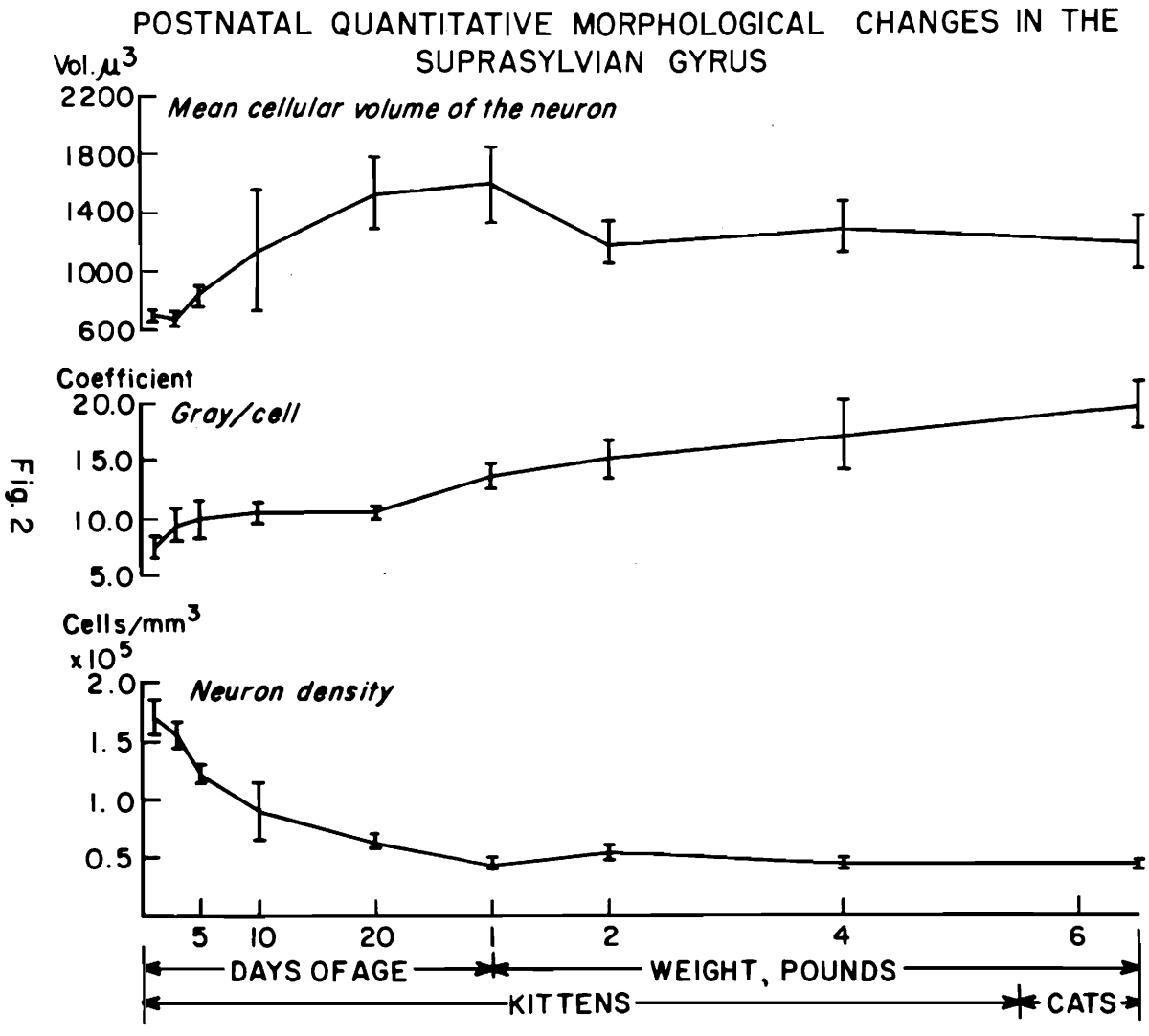

POSTNATAL QUANTITATIVE MORPHOLOGICAL CHANGES IN THE SUPRASYLVIAN GYRUS
Vol. μ3
2200
1800
1400
1000
600
Mean cellular volume of the neuron
Coefficient
20.0
15.0
10.0
5.0
Gray/cell
Cells/mm3
x10^5
2.0
1.5
1.0
0.5
Neuron density
5
10
20
1
2
4
6
DAYS OF AGE
WEIGHT, POUNDS
KITTENS
CATS

Fig. 2

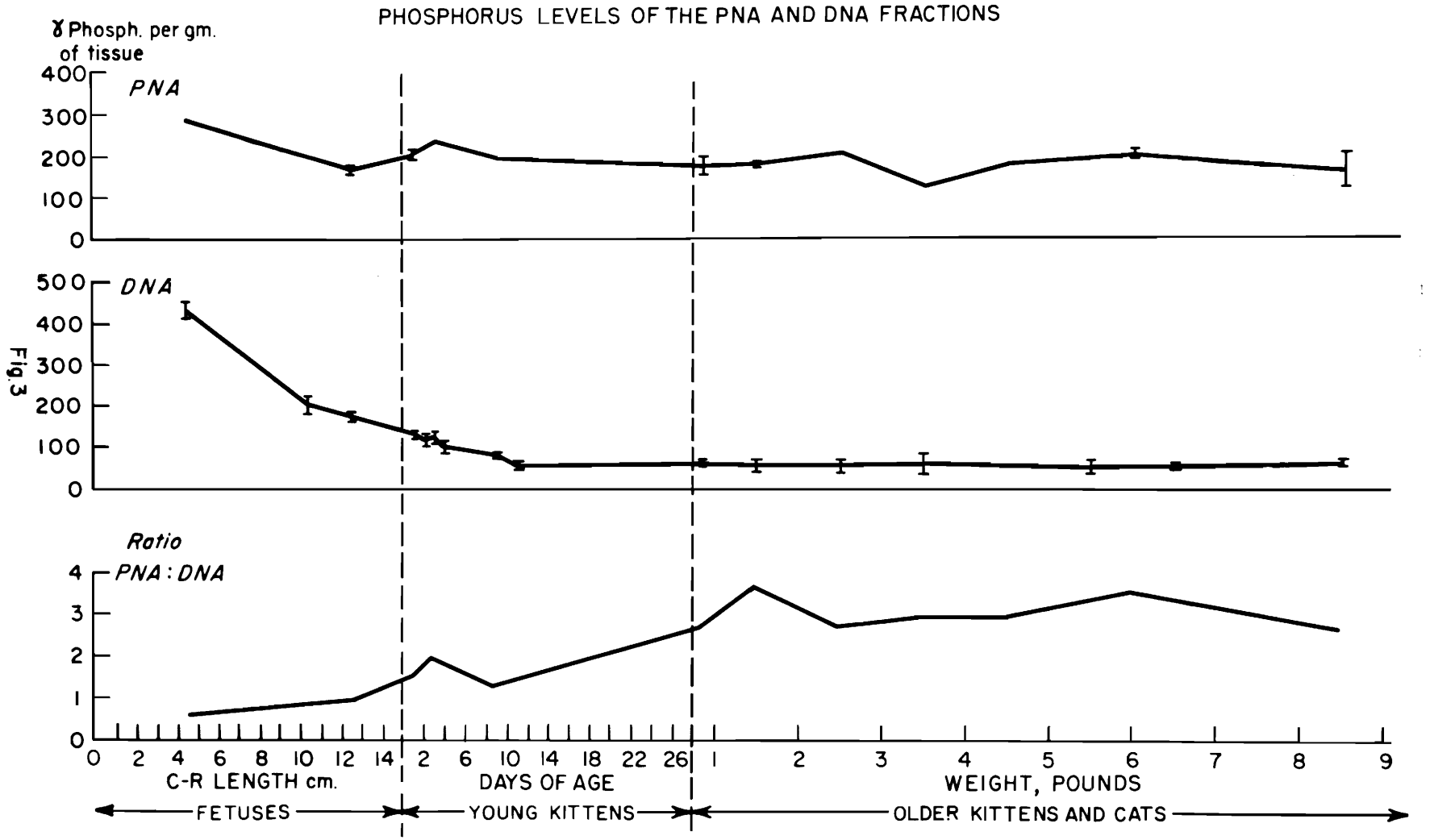

PHOSPHORUS LEVELS OF THE PNA AND DNA FRACTIONS
γ Phosph. per gm. of tissue
PNA
400
300
200
100
0
DNA
500
400
300
200
100
0
Ratio
PNA : DNA
4
3
2
1
0
0 2 4 6 8 10 12 14
C-R LENGTH cm.
2 6 10 14 18 22 26
DAYS OF AGE
1 2 3 4 5 6 7 8 9
WEIGHT, POUNDS
FETUSES
YOUNG KITTENS
OLDER KITTENS AND CATS

Fig. 3

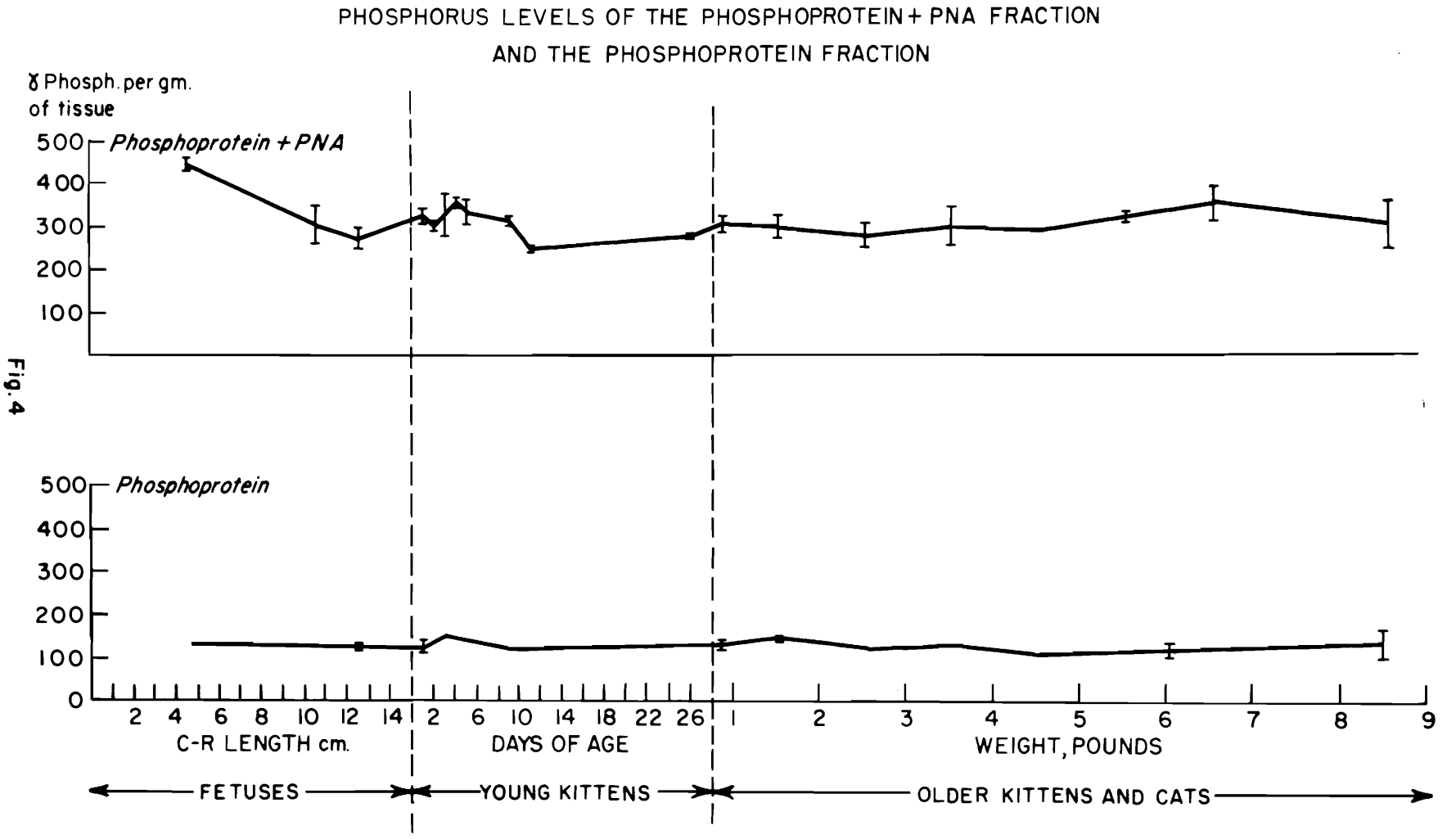

PHOSPHORUS LEVELS OF THE PHOSPHOPROTEIN + PNA FRACTION
AND THE PHOSPHOPROTEIN FRACTION
γ Phosph. per gm.
of tissue
Phosphoprotein + PNA
500
400
300
200
100
Phosphoprotein
500
400
300
200
100
0
2 4 6 8 10 12 14
C-R LENGTH cm.
2 6 10 14 18 22 26
DAYS OF AGE
1 2 3 4 5 6 7 8 9
WEIGHT, POUNDS
FETUSES
YOUNG KITTENS
OLDER KITTENS AND CATS

Fig. 4